

Identification and Quantitative Analysis of Marijuana

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1. Background

Marijuana (cannabis) is a common drug found in majority of drug cases. Marijuana consist tetrahydrocannabinol (THC) in both the male and female plants. THC is found in all parts of the plant in varying concentrations.

Marijuana samples will be tested and analyzed by four of the following elements macroscopic and microscopic analysis, Modified Duquenois-Levine Test and GC. Each of the four elements must yield positive characteristic results to identify samples as marijuana. If any of the four elements are not met or samples weighing over 30 pounds, then confirmatory analysis by GC/MS must be performed. Quantitative analysis will be performed on hashish samples weighing equal or greater than 28 grams.

2. Objective

The objective of this SOP is to establish guidelines to be used for the analysis of a sample that may contain marijuana.

3. Scope

This SOP is to be used by the laboratory staff of the Division of Analytical Chemistry at William A. Hinton State Laboratory Institute in Boston, MA.

4. Responsibility

Chemists are responsible for acquiring glassware, preparing chemical reagents and standards, sample analysis, and reporting. Chemists also perform instrument calibrations, maintenance and troubleshooting, ordering of supplies and other necessary tasks related to this analysis.

Laboratory Supervisors ensure that chemists are following this SOP. They may perform the duties of the chemists and must review raw data and reports generated by chemists. The Supervisor may advise the chemists of alternative testing methods. They ensure that quality control measures are within acceptable limits and determine when corrective actions are needed. They coordinate proficiency testing (PT), reporting and distribution of PT results. They oversee sample results distribution to outside agencies.

Directors ensure that the SOP is being followed and reviewed on a regular basis. They provide approval of standard operating procedures and review quality control documentations.

5. Related Documents

Drug Enforcement Administration, "Basic Training Program for Forensic Drug Chemists," Drug Enforcement Administration.

Mills III, Terry et al, "Instrumental Data for Drug Analysis," 3rd ed., 6 vols., New York: CRC Press, 2006.

Moffat, A.C. et al, "Clarke's Isolation and Identification of Drugs," 2nd ed., London: The Pharmaceutical Press, 1986.

Moffat, A.C. et al. "Clarke's Analysis of Drugs and Poisons," 3rd ed., London: The Pharmaceutical Press, 2004.

Saferstein, Richard, "Forensic Science Handbook," New Jersey: Prentice Hall, 1988.

Scientific Working Group for the Analysis of Seized Drug, revision 2011

AOAC

6. Definitions

GC: Gas Chromatography

GC/MS: Gas Chromatography/Mass Spectrometry

7. Supplies, Equipment & Reagents

Supplies

Porcelain dish

Hot Plate

Culture tubes

Volumetric Pipettes

Tweezers

Volumetric Flasks

Fixed or Adjusted Pipette

Weighing dish

Weighing paper

GC vials with Teflon caps

Equipment

Analytical Balance

Stereomicroscope

Auto sampler

GC

GC/MS

Reagents

Vanillin

Acetaldehyde

Hydrochloric Acid

Chloroform

Methanol

Petroleum Ether

8. Safety

Due to the potential hazards, appropriate precautions should be taken as necessary. This includes, but is not limited to, the use of fume hoods, gloves, masks and safety glasses.

Lab coats are to be worn at all times in the unit, unless performing administrative duties.

9. Reagent Preparation

Modified Duquenois Levine Reagent

Dissolve 4.0g of Vanillin in 2.5ml of Acetaldehyde and bring to volume with 200ml of 95% Ethanol. Mix the solution until completely dissolved.

Methadone Internal Standard (ISTD) Stock

A. Dissolve 25mg of Methadone into a 500ml volumetric flask. Bring to volume with methanol. Mix the solution until completely dissolved. (500ml = 0.50mg/ml ISTD)

- B. Remove 50ml of Methadone ISTD stock using a volumetric pipette into a 100ml amber bottle. (50ml = 0.50mg/ml ISTD)
- C. The remaining stock solution from A will be brought to volume with 500ml of methanol. (500ml = 0.45mg/ml ISTD)

THC Standards

THC Stock Solution

Pour 2 vials of Δ^9 THC (10mg/ml) reference standard into a tube. Mix the solution.

A. Concentration 1.0mg/ml THC with 0.45mg/ml ISTD

Remove 1.0 ml of Δ^9 THC standard into a 10ml volumetric flask and bring to volume with 0.50mg/ml ISTD methadone stock solution (B).

B. Concentration 0.40mg/ml THC with 0.45mg/ml ISTD

Remove 4 ml of 1.0mg/ml THC solution into a 10ml volumetric flask and bring to volume with 0.45mg/ml ISTD methadone stock solution (B).

C. Concentration 0.20mg/ml THC with 0.45mg/ml ISTD

Remove 1.0 ml of 0.40mg/ml THC solution into a 10ml volumetric flask and bring to volume with 0.45mg/ml ISTD methadone stock solution (B).

11. Weighing and Sampling Plan

(still trying to decipher the policy, depending on chemist)

12. Procedure

- A. Document observations on the Drug Analysis Form noting the number, type (e.g. cigarette, vegetable matter) and marking of all items.
- B. Determine the appropriate sampling plan and weight for each case.
- C. Remove a portion of the sample and place it into a porcelain dish.
- D. Macroscopic Identification
 - i. View the sample by a visual examination.
 - ii. Identify the gross morphological characteristics that may be observed including palmate arrangements, pinnate appearance, serrated edges of the leaflets, buds (with or without seeds) and if present stems and stalks.
 - iii. Positive macroscopic examination will be recorded on the Drug Analysis Form by the use of a plus (+). The result is considered positive when sufficient characteristics are observed and are specified in the notes. Negative observations will be recorded by the use of a negative (-).
- E. Microscopic Identification
 - i. View the sample at varying magnifications (approx. 10x – 40x) using a stereomicroscope.
 - ii. Identify the cystolithic hairs. Cystolithic hairs are unicellular, sharply pointed curved, conical trichome with enlarged bases that contain deposits of calcium carbonate. The shapes of these hairs resemble “bear claws.” They are found in greater abundance on the upper side of the leaf.

- iii. Identify the glandular hairs. Glandular hairs are multicellular with conical trichomes that are long and slender. These hairs have a shiny appearance and a sticky touch due to the resin. The glandular hairs form on the surface of the leaf or flowering tops.
- iv. Positive microscopic examination will be recorded on the Drug Analysis Form by the use of a plus (+). The result is considered positive when sufficient characteristics for both the cystolithic and glandular hairs are observed and are specified in the notes. Negative observations will be recorded by the use of a negative (-).

F. Modified Duquenois Levine Color Test

- i. Extract sample with petroleum ether in a porcelain dish.
- ii. Using a hot plate, evaporate the solution to dryness.
- iii. Add 2ml of Duquenois reagent and stir to bring the residue into the solution.
- iv. Add 2ml of concentrated Hydrochloric Acid stir and let stand for a few minutes. A color change will develop. A positive reaction to the Duquenois portion is a blue/purple color.
- v. Transfer the colored solution to a labeled culture tube and shake with 2ml of chloroform. Two discernable layers will form. For a positive reaction to the Levine portion, the bottom layer will turn a pink/purple in the presence of marijuana.
- vi. Positive Duquenois Levine Color test will be recorded on the Drug Analysis Form by documenting the actual color/s observed. The result is considered positive when both the Duquenois and Levine portions are observed and are specified in the notes. Negative observations will be recorded by stating no reaction present.

G. Gas Chromatography

- i. GC analysis will be performed on all suspected marijuana cases.
- ii. Weigh 25mg of sample and place into a labeled culture tube.
- iii. Dispense 5ml of appropriate solvent (i.e. methanol, chloroform, petroleum ether) into the labeled culture tube.
- iv. Transfer 2ml of the solution into a labeled GC vial and cap tightly.
- v. Initiate auto sampler sequence using the Routine method running a blank solvent between each unknown sample and reference standard.
- vi. Compare retention time of the each sample with the reference standard. Also check the chromatograph to determine if the sample needs to be diluted or concentrated.
- vii. Positive GC analysis will be recorded on the Drug Analysis Form by the use of a plus (+). The result is considered positive when the retention time of the sample and the reference standard meet the laboratory criteria and are specified in the notes. Negative observations will be recorded by the use of a negative (-).

H. Gas Chromatography/Mass Spectrometry

- i. Confirmatory test will be performed if the results of any of the 4 prior tests are inconclusive or negative or if the net weight of the sample is equal or greater than 30 pounds.
- ii. Confirmatory analysis can be performed using the GC vial from the previous section (G).
- iii. Initiate auto sampler sequence using the THC method running a blank solvent between each unknown sample and reference standard.
- iv. Compare retention time and ion spectra of the each sample with the reference standard.
- v. Document the date analyzed and results of the GC/MS onto the MS Tracking Sheet, Drug Analysis Form and Control Card.

I. Residual Samples

- i. Document observations on the Drug Analysis Form noting the number, type of device and marking of all items.
- ii. Attempt to scrape or remove sample from the device and place into a porcelain dish.
- iii. Proceed to perform the Macroscopic Identification. (see 11D)
- iv. Proceed to perform the Microscopic Identification. (see 11E)
- v. Rinse the device containing the sample with 1-2ml of the appropriate solvent (i.e. methanol, chloroform, petroleum ether.)
- vi. Transfer some solvent into a residue for GC and GC/MS analysis.
- vii. Using a hot plate, evaporate the remaining solution to dryness.
- viii. Proceed to perform the Modified Duquenois Levine Color Test. (see 11F.)
- ix. Proceed to perform the GC analysis. (see 11G.)
- x. Proceed to perform the GC/MS analysis. (see 11H.)

J. Hashish

- i. Quantitative analysis will be performed on suspected Hashish sample that are equal to or greater than 28 grams.
- ii. Document observations on the Drug Analysis Form noting the number, type and marking of all items
- iii. Obtain and document the gross and net weight of the sample. Determine the appropriate sampling plan for each case.
- iv. Remove a portion of the sample and place it into a porcelain dish.
- v. Proceed to perform the Macroscopic Identification. (see 11D)
- vi. Proceed to perform the Microscopic Identification. (see 11E)
- vii. Proceed to perform the Modified Duquenois Levine Color Test. (see 11F.)
- viii. Proceed to perform the GC analysis. (see 11G.)
- ix. Proceed to perform the GC/MS analysis. (see 11H.)

1. THC Quantitative

- a. Weigh and crush up 20mg of sample and place into a culture tube with 10ml of 0.45mg/ml ISTD.
- b. Allow the sample to soak overnight in the refrigerator.
- c. Transfer 2ml of the solution into a labeled GC vial and cap tightly. Prepare two separate GC vials.

- d. Initiate auto sampler sequence using the THC Quant method running a blank solvent between each sample, standards and control.
- e. Run the primer (0.20mg/ml control).
- f. Run the calibrator (1.0mg/ml STD).
- g. Run sample/s, standards and controls.
- h. Report as Δ^9 THC > 2.5 %

10. Purity Calculations

$$\% \text{ Drug} = \frac{(\text{STD}) \times R2 \times V}{R1 \times W} \times 100$$

(STD) = concentration of calibration standard in mg/ml

$$R2 = \frac{\text{peak area (height) of sample}}{\text{peak area (height) of internal standard}}$$

$$R1 = \frac{\text{peak area (height) of standard}}{\text{peak area (height) of internal standard}}$$

V = volume of internal standard solution used in ml

W = sample weight in mg

13. Documentation

- A. All results will be documented on the Drug Analysis Form.
- B. All raw data will be generated and filed according to the laboratory policy.
- C. A certificate of analysis will be generated for each lab number which will document the results.

14. Attachments